

An investigation of anticoccidial veterinary drugs as emerging organic contaminants in groundwater

D. Mooney, K.G. Richards, M. Danaher, J. Grant, L. Gill, P.-E. Mellander, C.E. Coxon



PII: S0048-9697(20)34645-3

DOI: <https://doi.org/10.1016/j.scitotenv.2020.141116>

Reference: STOTEN 141116

To appear in: *Science of the Total Environment*

Received date: 28 May 2020

Revised date: 14 July 2020

Accepted date: 18 July 2020

Please cite this article as: D. Mooney, K.G. Richards, M. Danaher, et al., An investigation of anticoccidial veterinary drugs as emerging organic contaminants in groundwater, *Science of the Total Environment* (2020), <https://doi.org/10.1016/j.scitotenv.2020.141116>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

## **An investigation of anticoccidial veterinary drugs as emerging organic contaminants in groundwater**

D. Mooney <sup>a, b, c\*</sup>, K. G. Richards <sup>c, d</sup>, M. Danaher <sup>b</sup>, J. Grant <sup>e</sup>, L. Gill <sup>c, f</sup>, P-E. Mellander <sup>g</sup>, and C.E. Coxon <sup>a, c</sup>

<sup>a</sup> Geology Department/ Trinity Centre for the Environment, School of Natural Sciences, Trinity College Dublin, Ireland

<sup>b</sup> Food Safety Department, Teagasc, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland

<sup>c</sup> Irish Centre for Research in Applied Geosciences (iCRAG), Ireland

<sup>d</sup> Environment, Soils and Land-Use Department, Teagasc Environment Research Centre, Johnstown Castle, Wexford, Ireland

<sup>e</sup> Statistics and Applied Physics, Research Operations Group, Teagasc, Ashtown, Dublin 15, Ireland

<sup>f</sup> Department of Civil, Structural and Environmental Engineering, Trinity College Dublin, Ireland

<sup>g</sup> Agricultural Catchments Programme, Teagasc Environment Research Centre, Johnstown Castle, Wexford, Ireland

\* Corresponding author: D. Mooney, Geology Department, School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland

Tel. +353 1 8059596; Fax. +353 1 8059500

Email address: mooneyd2@tcd.ie

### **Abstract**

Intensification of the food production system to meet increased global demand for food has led to veterinary pharmaceuticals becoming a critical component in animal husbandry. Anticoccidials are a group of veterinary products used to control coccidiosis in food-producing animals, with primary prophylactic use in poultry production. Excretion in manure and subsequent land-spreading provides a potential pathway to groundwater. Information on the fate and occurrence of these compounds in groundwater is scant, therefore these substances are potential emerging organic contaminants of concern. A study was carried out

to investigate the occurrence of anticoccidial compounds in groundwater throughout the Republic of Ireland. Twenty-six anticoccidials (6 ionophores and 20 synthetic anticoccidials) were analysed at 109 sites (63 boreholes and 46 springs) during November and December 2018. Sites were categorised and selected based on the following source and pathway factors: (a) the presence/absence of poultry activity (b) predominant aquifer category and (c) predominant groundwater vulnerability, within the zone of contribution (ZOC) for each site. Seven anticoccidials, including four ionophores (lasalocid, monensin, narasin and salinomycin) and three synthetic anticoccidials (amprolium, diclazuril and nicarbazin), were detected at 24% of sites at concentrations ranging from 1 to 386 ng L<sup>-1</sup>. Monensin and amprolium were the two most frequently detected compounds, detected at 15% and 7% of sites, respectively. Multivariate statistical analysis has shown that source factors are the most significant drivers of the occurrence of anticoccidials, with no definitive relationships between occurrence and pathway factors. The study found that the detection of anticoccidial compounds is 6.5 times more likely when poultry activity is present within the ZOC of a sampling point, compared to the absence of poultry activity. This work presents the first detections of these contaminants in Irish groundwater and it contributes to broadening our understanding of the environmental occurrence and fate of anticoccidial veterinary products.

**Keywords:** emerging contaminants; ionophores; coccidiostats; feed additives; poultry production, groundwater

## 1. Introduction

According to the Groundwater Directive 2006/118/EC (European Parliament, 2006), groundwater is the largest body of fresh water within the European Union (EU), with up to 75% of EU residents depending on groundwater as a source of drinking water. In Ireland, approx. 26% of the public and private drinking water supply is provided by groundwater sources, with more localised regions relying on groundwater for up to 75% of their needs

(EPA, 2010). There are approx.172,000 households (equating to 17% of the national population) that obtain their drinking water supply from private groundwater sources and springs (CSO, 2017), which do not fall under the regulations implementing the EU Directive on water for human consumption (98/83/EC) in Ireland (Government of Ireland, 2014)). Most of these private water supplies are more susceptible to contamination given that the onus for monitoring and maintenance is with the individual owners (EPA, 2010). Poorly sited and/or constructed supplies are likely to have an increased risk of contamination (Misstear et al., 2017, Gill et al., 2018). Groundwater also plays an essential role in contributing to and maintaining surface water flow, and as a result, groundwater quality issues are often reflected in surface water bodies and wetlands. This further magnifies the importance and need for groundwater protection and risk assessment, both for the benefit of drinking water consumers, but also for a wide range of groundwater dependent ecosystems.

An Environmental Protection Agency (EPA) of Ireland groundwater pressure risk assessment report indicated that nutrient pressures from agricultural activities (including livestock farming, arable activities and intensive enterprises) and usage of dangerous substances such as agrochemicals, are the most widespread, and nationally significant, anthropogenic pressure on groundwater in Ireland (Clabby et al., 2008). In recent years, synthetic organic compounds, often known as emerging organic contaminants (EOCs), are becoming more of a growing international concern regarding their occurrence in, and contamination of, groundwater bodies (Lapworth et al., 2012, Postigo and Barcelo, 2015). Veterinary pharmaceuticals have become a critical component in Irish agriculture because of the ever-increasing demands on the production systems to provide more foods, particularly of animal origin. The administration of such veterinary products can potentially lead to their occurrence in groundwater once excreted by the animal. As a result, all veterinary pharmaceuticals are potentially emerging groundwater contaminants of concern, depending on their fate and eco-

toxicological behaviour in the environment (Lapworth et al., 2012).

In recent analytical method development work by this research group, 26 anticoccidials (6 ionophores and 20 synthetic anticoccidials (often referred to as chemical coccidiostats)) were selected as a group of veterinary products/feed additives of interest, due to their potential to be emerging groundwater contaminants of concern (Mooney et al., 2020). These compounds were selected due to the current lack of information on their environmental occurrence and fate, with more emphasis and priority given to the 11 compounds currently licensed as feed additives in the EU (European Parliament, 2003) (Table 1). To broaden the scope, and to enhance the applicability of the methodology, some additional compounds that are authorised outside of the EU (e.g. in the USA) were also included in the original method development work. All 26 compounds (and their abbreviations) investigated in this study are listed in Table 1, with compounds grouped as ionophores or synthetic anticoccidials.

While there are 11 licensed anticoccidial feed additives in the EU, the usage (and therefore potential environmental source) of anticoccidials differs from one country to another; for example, in Denmark only four of the six licensed ionophores are used as feed additives (Bak and Björklund, 2014). In Ireland, the majority of licensed anticoccidials are used exclusively in poultry species, with the primary source in the environment likely to be a consequence of their prophylactic use in intensive poultry production. A very limited number of anticoccidials (e.g. diclazuril and toltrazuril) are also used in Ireland as therapeutics in other production systems such as cattle and sheep, however to a much lesser extent than as feed additives.

The introduction of anticoccidials into the environment is potentially from direct excretion of faeces and/or urine on land, spreading of manure and slurry collected from the production/housing units, or because of point source contamination at or near the production

facilities (Boxall, 2010, Alonso et al., 2019). There is a lack of information on anticoccidials and the factors that can influence their entry to the environment; there is a shortage of data in international literature on the metabolism, and excretion, of anticoccidials following administration, while information on the attenuation and degradation processed for the contaminants, once in the environment, is scant. Of the limited information that is available, several anticoccidials (e.g. lasalocid (LAS) and diclazuril (DICLAZ)) can be excreted in sizeable amounts (up to 95% of administered dose) as un-metabolized active substances (EFSA, 2004, Hansen et al., 2009a).

Movement of these contaminants to groundwater can depend on different environmental and hydrogeological factors such as land use, soil properties, geological and hydrogeological properties and climate (Essaid et al., 2015). Climatic conditions, particularly the timing of effective rainfall, are an important factor which can produce temporal variability in contaminant transport (Harman et al., 2011). The intrinsic physicochemical properties of the individual compounds themselves are also vital (Table 1). While in transport, contaminants are subject to several complex physical, chemical and biological transformation processes that can provide attenuation, depending on the pathway taken (Arias-Estévez et al., 2008). Based on the physicochemical properties of the anticoccidials (mostly highly hydrophobic, with high organic carbon sorption coefficients), the most important of these environmental factors are soil and Quaternary deposit properties (such as pH, texture, structure, organic content, permeability and thickness), with adsorption to soil likely to be a significant attenuation process as these contaminants move through the unsaturated zone to groundwater. The pH of both soil and water has been shown to play a critical role in the transport of anticoccidials, particularly the ionophores, with different chemical speciation occurring at varying pH (Hansen et al., 2009a, Alonso et al., 2019). Given the potential for the strong adsorption of anticoccidials to soils and sediment, there is the potential for transport of the

contaminants to groundwater via colloidal transport (Foster and Chilton, 1991) through preferential flow pathways, therefore increasing the vulnerability of groundwater to these contaminants. In a study in Brazil, Yopasá-Arenas and Fostier (2018) produced vulnerability maps for a qualitative approach to risk assessment of the exposure of Brazilian soils and groundwater to anticoccidials (monensin (MON) and salinomycin (SAL)) and antimicrobial growth promoters, which indicated that groundwater was more vulnerable than soil.

There is also a dearth of information on the occurrence of anticoccidials in environmental waters. Mooney et al. (2020), attributed this to the lack of suitably sensitive and comprehensive analytical methods. Most published environmental detections of anticoccidials relate to the ionophores (Watanabe et al., 2008, Bartelt-Hunt et al., 2011, Sun et al., 2013, Bak and Björklund, 2014), with very few relating to synthetic anticoccidials (Song et al., 2007, Iglesias et al., 2012). Some studies have reported on anticoccidial occurrence in surface waters and agricultural runoff (including agricultural land drains) (Kim and Carlson, 2006, Song et al., 2010, Iglesias et al., 2012, Sun et al., 2013, Bak and Björklund, 2014, Alonso et al., 2019) or in solid agricultural samples (including soil, sediment, manure/litter) (Biswas et al., 2012, Sun et al., 2013), but very few have reported occurrence in groundwater bodies (Watanabe et al., 2008, Bartelt-Hunt et al., 2011). MON, SAL and NAR (narasin) are by far the most commonly detected ionophores in environmental water samples, with detected concentrations in groundwater and surface waters between 2 and 390 ng L<sup>-1</sup>. However, some studies have reported concentrations of the order of thousands of nanogram per litre (i.e. microgram per litre) e.g. 2350 ng L<sup>-1</sup> of MON detected in groundwater (Bartelt-Hunt et al., 2011) and 9022 ng L<sup>-1</sup> of SAL in run-off (Sun et al., 2013).

The key concerns over the occurrence of anticoccidials in groundwater primarily relate to consumption in drinking water, and environmental effects on non-target organisms. Boxall

(2010) included ten anticoccidial compounds (including MON, SAL, LAS, NICARB (nicarbazin) and DICLAZ ) on a list of 56 veterinary medicines which are high priority in the environment, based on usage, amounts likely to enter the environment, and their environmental toxicity. Capleton et al. (2006) carried out a similar study but looked at the risk of indirect exposure and toxicity to humans, with several anticoccidials (MON, SAL and toltrazuril (TOL)) also classified as high priority veterinary medicines in need of detailed risk assessments. Both studies cite the lack of complete information on usage as a limitation to the study and risk assessment. The primary concern over human consumption is the long term exposure to low levels, leading to potential chronic toxicity (Biswas et al., 2012, Roila et al., 2019). Some acute effects of anticoccidials, such as the ionophores, have been observed in humans, with muscle cell necrosis occurring as a result of the increased intracellular concentrations of sodium and calcium, which is transported across membranes in ionophore complexes (Dorne et al., 2013). Previously documented clinical symptoms include skeletal and cardiac muscle loss and/or weakness, with some cases resulting in organ failure leading to death (Caldeira et al., 2001). However, all such instances have occurred in cases of unintended ingestion of the substances, with exposure to concentrations much higher than those expected in environmental waters. Recently concerns have been raised that the use of anticoccidials in feed could cause the development of bacteria with antimicrobial resistance in both humans and animals (VKM, 2015).

The aim of this work was to investigate the occurrence of a wide range of anticoccidial compounds in Irish groundwaters, with a focus on karstic and fractured bedrock aquifers, which dominate the hydrogeology of Ireland. This work specifically aimed to determine the frequency of occurrence of the different anticoccidial compounds, and to investigate the drivers behind detections (such as land-use and hydrogeological characteristics) to help advance our understanding of their fate.



## 2. Materials and Methods

### 2.1 Sampling sites

#### 2.1.1 Sampling Pool

Sampling points (referred to as monitoring points, MPs) were selected from a pool of over 320 pre-existing, groundwater sampling sites from across the Republic of Ireland. This extensive list of potential MPs incorporated >280 MPs included as part of the national groundwater quality monitoring programme for the E.U. Water Framework Directive (WFD), implemented in Ireland by the EPA (EPA, 2019). The remaining sites comprised private and/or semi-private group water schemes (GWSs), sampled in coordination with the National Federation of Group Water Schemes (NFGWS). The EPA groundwater monitoring network mainly comprises public and private drinking water abstraction points, represented by production boreholes (BH) or springs (SP) with large abstraction and discharge rates. GWSs vary in size and capacity, with individual schemes providing for a few houses, up to hundreds of houses. The EPA national groundwater monitoring programme was designed to represent the variation in hydrogeology and pressures across the various groundwater bodies (Craig et al., 2006).

#### 2.1.2 Zone of Contribution

A zone of contribution (ZOC) is described as the catchment area that contributes water to a borehole or spring (Kelly, 2010, Misstear et al., 2017). Delineation of such a ZOC provides the area boundary, within which, the anthropogenic activities and geological and hydrogeological properties can influence the water quality at the associated MP. ZOC's for all the aforementioned potential sampling sites have previously been delineated using the approach summarised by Kelly (2010). For this study, ZOC data were obtained in the form of shapefiles (EPA, 2018), which were manipulated using ArcGIS 9.3 (Geological Information System) for site classification.

### 2.1.3 Selection Process

Sites were classified based on land use and physical hydrogeological properties, selected to account for different source and pathway factors. The three key site characteristics selected were: (a) aquifer category/flow regime, (b) groundwater vulnerability (both pathway factors) and (c) poultry activity (source factor). Aquifer category was determined using the Geological Survey of Ireland classification system, with aquifer classes divided into three main groups based on their resource potential (Regionally Important, Locally Important or Poor Aquifers), and further subdivided based on the openings through which groundwater flows (DELG/EPA/GSI, 1999) (Table 2). These 11 GSI aquifer classes were also amalgamated into four WFD flow regime categories (Working Group on Groundwater, 2001). Groundwater vulnerability, defined as the intrinsic geological and hydrogeological characteristics that determine the ease with which groundwater may be contaminated by human activities (DELG/EPA/GSI, 1999), was classified into four major categories: Extreme (subdivided into X and E), High (H), Moderate (M) and Low (L), based primarily on the subsoil permeability and thickness (depth to bedrock). Poultry source factors were taken into consideration when selecting sites by using data on poultry farms and poultry manure spreading within the ZOC of each MP, from a limited dataset provided by the Department of Agriculture, Fisheries and Marine (DAFM) (unpublished data, see Supplementary File 1). Poultry activity was classified by the presence or absence of poultry activity within the ZOC, with poultry activity defined as poultry farms and/or manure spreading.

Data for each of these site characteristics were extracted in ARCGIS using the tabulate intersection tool, which computes the intersection between two feature classes and cross-tabulates the area of the intersecting features (ESRI, 2016). This tool outputs the percentage area of the ZOC accounted for by the different classes within each property layer (except for poultry farms, presented as no. of farms within the ZOC). Each MP was characterised based

on the most predominant class of each physical site characteristic within the ZOC. Predominance, in most cases, was assigned to the class which accounted for >50% of the ZOC area.

#### 2.1.4 Final Sampling Sites

For the overall sampling campaign, 109 MPs, comprising 63 BHs and 46 springs, were selected from across 25 of the 26 counties in the Republic of Ireland. Samples were collected during November and December 2018, during a period with active groundwater recharge conditions. An approximate 70/30 ratio was adopted between MPs with poultry activity present vs. absent, with 68% classified as having poultry activity present. Table 3 below summarises the spread of the 109 MPs, selected across the different bedrock aquifer categories (GSI), and the three bedrock aquifer flow regime classes. Overall 50% of the total MPs selected fell within the karstic flow regime, with 17% and 33% of sites falling under the productive fractured and poorly productive categories, respectively. This spread achieves a representative sample of each of the three bedrock aquifer flow regimes, with a focus on productive aquifers, in broad agreement with the proportions of groundwater bodies in Ireland within each regime (Daly, 2009). Table 3 also shows the spread of the number of MPs across the five different groundwater vulnerability classes.

#### 2.2 Sample Collection, Storage and Chemical Analysis

Raw, unfiltered, groundwater samples were taken in accordance with ISO EN 5667 standards (NSAI, 2009, NSAI, 2018) and EPA protocols. Samples were collected in pre-cleaned amber borosilicate glass bottles, with a 1L sample enough to allow for initial analysis and further repeat analysis in duplicate. Depending on the MP, sampling was carried out directly from the source, or via pre-existing distribution pump houses (where the source was not accessible or enclosed). As a result, sampling was carried out by one of three techniques: (a) directly into the sampling container (e.g. from a tap or shallow spring); (b) using a discrete depth

sampler, in this case a closed bailer device or (c) by pump using Teflon tubing. BHs, lacking a raw water sampling tap, with standing water levels >2m below ground level were sampled by pump (WaSP Five Stage 12V Submersible Pump (In-Situ, Europe, Worcestershire, UK)) and those with <2m were sampled by bailer (100mL or 250mL). Prior to collection, the sampling bottle was rinsed (a minimum of three times) with the source water. For sites included as part of the EPA monitoring network (98 sites, as listed in Supplementary File 2), groundwater physicochemical “field” parameters (listed in Supplementary File 1 Table S5) were measured and the sample was taken after four consecutive, stable readings.

Once collected, samples were transported to the laboratory under chilled conditions (<4°C), arriving at the laboratory no later than 24 hours after collection and remained in storage at 4°C until analysis, within 10 days of collection. Analysis of groundwater samples was carried out according to the method previously developed and validated by Mooney et al. (2020), for the determination of 26 anticoccidial compounds (as listed in Table 1) in environmental waters. An organic modifier (7.5 mL Methanol) was added to samples (250 mL) to assist with desorption of residual analytes potentially sorbed on the sample container, with the modified samples subsequently pH adjusted (pH 8.5) and extracted using Enviro Clean HL-DVB solid phase extraction (SPE) cartridges (200 mg, 6 mL), packed with glass wool. The SPE cartridges were eluted (MeOH:MeCN:EtOAc, 40:40:20, v/v, 12 mL) and evaporated (0.5 mL) for final instrumental analysis by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). All analytes were chromatographically separated using a Zorbax Eclipse Plus Phenyl-Hexyl Rapid Resolution HD analytical column on an Agilent 1290 Infinity™ II UHPLC system and detected using an AB Sciex 6500+ quadrupole linear ion trap (QTRAP) mass spectrometer. This method was deemed fit for purpose for the confirmatory analysis of all analytes except toltrazuril and its two transformation products toltrazuril sulfoxide and toltrazuril sulphone, which were included

in this study for screening only. All samples were initially analysed singly, with any samples with detections further repeated in duplicate, with the result reported as the mean concentration of the triplicate analyses.

Negative control samples were used to produce QC Trip (Field) blanks, also as described by Mooney et al. (Mooney et al., 2019, 2020). QC trip blanks were transported to the sampling sites, exposed to the environment while sampling, and transported back to the laboratory alongside normal samples for analysis, to demonstrate a lack of cross contamination in the field and during transportation. Fortified QC field samples were not feasible, given sampling was carried out by varying personnel from different organisations. There were no measurable detections of any target compounds found in field blanks during this campaign.

The 98 EPA samples underwent additional analysis at the EPA laboratories for several water quality parameters including the major ions, nutrients, metals and faecal microorganisms using standard methods. A full list of these parameters is provided in Supplementary File 1 Table S5, which also highlights the method of analysis and relevant detection capabilities. Results of these analyses were provided by the EPA (unpublished data) and used to investigate any association with detection of the anticoccidial contaminants that could be used as a surrogate indicator for potential anticoccidial contamination of groundwater.

### *2.3 Statistical Analysis*

While the datasets used below for statistical analysis cannot be claimed to be free of bias because of the non-random sampling, the authors contend that the sample selection corresponds to an expert sample and therefore the statistics calculated from the data contains useful information on the processes that were being examined. The scope of any statistical inference should be viewed as exploratory in the sense that it could form a basis for designing future studies, rather than claiming firm associations from these data.

### 2.3.1 Additional dataset preparation for statistical analysis.

Several other physical site properties were also used for statistical analysis, to investigate any association between anticoccidial detections, and these site characteristics. These properties and the classes into which they were subdivided, are listed in Table 2, and are described in more detail in Supplementary File 1. The predominant class of each of these properties were determined using the same process described in Section 2.1.3, with the predominant class within the ZOC of each MP recorded (Supplementary File 2) and input for statistical analysis. In order to provide enough observations for a more meaningful statistical analysis, and to allow a more stable, accurate logistic regression analysis, several datasets with many individual, were amalgamated into fewer categories/classes (preferably <10 classes), as also described in supplementary file 1. Assignment of the predominant class for a number of site characteristics was problematic, therefore statistical analysis was also carried out on the relative percentage data, of each class within the ZOC.

### 2.3.2 Site physical characteristics

Testing for association between detection of anticoccidials and physical site characteristics was carried using SAS 9.4 (SAS Institute Inc, 2014). Since the overall prevalence of detections above the LOQ was very low, resulting in biased estimation of means (Helsel, 2011), a positive result in any test for presence of the contaminants was coded as a detection and the resulting classification of each sample as Detect/Non-detect was analysed as a binary response. The Logistic procedure in SAS was used to fit a regression to quantify the relationship between the binary response and the explanatory variables (Supplementary File 2). Initially a variable selection procedure was used to identify variables associated with detection of contaminants and then the marginal effect of each explanatory variable was tested. Relationships were quantified using odds ratios and their 95% Wald confidence limits. Sampling MP type was included as a factor in the modelling to correct for any possible

confounding of effects. Where the regression failed to converge, Fisher's Exact Test with Monte-Carlo simulation was used to test for independence of the explanatory variable and detection.

Several definitions of detection were analysed as follows; (a) any detection across the full set of 26 contaminants, (b) detection of an ionophore compound and (c) detection of a synthetic anticoccidial. In addition, MON was also analysed individually, given it is the most commonly reported anticoccidial detected in groundwater. All analyses were repeated for a subset of the data (74 sampling points) where only those observations with poultry "present" within the ZOC of the sampling point, were included. This approach was deemed reasonable given MPs with confirmed contaminant sources were more informative in distinguishing between MPs that have a source of and detection of anticoccidials, compared to MPs that had a source, but non-detection, of anticoccidials.

### 2.3.3 Water quality parameters

A similar approach was used to test for relationships with anticoccidial occurrence and water quality parameters, but on a reduced number of MPs ( $n=98$ ), given such data was only available for the EPA MPs. The analysis was problematic because of varying degrees of censoring of the quality parameters at the limit of quantitation (LOQ). Water quality parameters with no censoring, and those with one to three values missing or censored, were straightforwardly modelled using logistic regression with detection/non-detection as the response. Measurements with very high levels of censoring ( $>70\%$ ) are very subject to bias in estimating mean values and so these were recoded as present/not-present and tabulated for testing against contaminant detections. Characteristics with intermediate levels of censoring were recoded and tested in the same way but were further explored. For these, substitution of zero and LOQ values was used to examine the maximum and minimum potential outcome differences and a nonlinear model was fitted that incorporated the calculation of the statistical

likelihood of censored data (Long, 1997). Dealing with the censoring in the explanatory variable was also problematic and rather than using a censored characteristic as an explanatory variable to model detections, detection and non-detection were treated as a grouping variable and the means for those two groups were compared. This allowed methods for censored responses to be used.

### 3. Results

#### *3.1. Groundwater occurrence—summary of anticoccidials detected*

Of the 109 MPs, 24% (26 sites) had a detection of at least one anticoccidial compound, with 7 out of the 26 different compounds detected throughout the campaign, at concentrations ranging from 1 to 386 ng L<sup>-1</sup>. Up to three different compounds were detected at any given MP. Table 4 outlines some summary statistics for the seven compounds detected, while Table 5 summarises the 26 sites that had anticoccidial detections, and the associated concentrations. Ionophore compounds were detected at 19.3% of sites, while synthetic anticoccidials were detected at 11% (Table 4). The ionophore MON was the most frequently detected anticoccidial, detected at 14.7% of the total sites (16 of 109). MON was detected at the highest concentration with one sample containing up to 386 ng L<sup>-1</sup>, however the overall median concentration amongst the 26 sites was much lower (17.5 ng L<sup>-1</sup>). The next most often detected compound was the synthetic anticoccidial amprolium (AMP), detected at 7.3% of sites at concentrations up to 49.8 ng L<sup>-1</sup>, with an overall median concentration of 11.9 ng L<sup>-1</sup>. The ionophore LAS was detected at 5.5% of sites, and at the lowest concentration throughout the study (1.9 ng L<sup>-1</sup>), however the overall median concentration (14.7 ng L<sup>-1</sup>) was similar to other ionophores. SAL had the overall lowest median concentration (9.8 ng L<sup>-1</sup>) detected across just 4% of the total sites.

#### *3.2 Analysis of factors controlling spatial distribution*



### 3.2.1 Occurrence and ZOC site characteristics

The geographical spread of the 109 MPs classified by MP type and detect/non-detect is shown in Figure 1 below. All sites with detections, except for two, were classified as having poultry activity present within their ZOC. BHs accounted for 65% of the sites with detections and anticoccidials were recorded at 27% of BHs. Figure 2 below gives an overview of the distribution of detections across the different classes of aquifer category, WFD flow regime, and groundwater vulnerability. These summary statistics do not indicate any clear relationship between detections and aquifer category, flow regime or groundwater vulnerability, with detections spread relatively even across the different classes of each of these three site characteristics. Statistical analysis further confirmed this observation, with no significant relationship shown (Supplementary Table S6).

Detections of (a) all anticoccidials, (b) ionophore compounds and (c) synthetic anticoccidial compounds were observed to be significantly ( $p < 0.05$ ) related, or almost significantly related ( $p < 0.06$ ), to several site characteristics, namely poultry activity, poultry farm density, poultry manure spreading and IFS (Type I) acidic/basic soils (Table 6). For relationships that were significant ( $p < 0.05$ ) the interpretation of the odds ratio likelihood outputs and the associated 95% confidence intervals are presented (Table 6). All other characteristics were not statistically related to anticoccidial occurrence, with a full summary of p-values for all characteristics, for each of the detection definitions provided in Supplementary File 1 Table S6. The Fisher Exact test returned a significant result for SIS soil association ( $p = 0.0335$ ) but interpreting the detail of the detected association is not practical because of the large number of classes explaining the detection and the resulting sparseness of the tabulation, including many zeros.

Focusing on MPs classified as “poultry present” produced similar results to the above for the different detect definitions, however, IFS Type I class (Acidic vs. basic soils) was

significantly related to detections of synthetic anticoccidials ( $p = 0.0268$ ), ionophores (0.0392), and anticoccidials collectively ( $p = 0.0183$ ). In all cases, detections were more likely in acidic soils (synthetic anticoccidials 7.7 times more likely, an ionophore 3.3 times more likely and anticoccidial 3.6 times more likely). These effects were confirmed by analysis of the percentage of each IFS Type 1 class, which indicated that a detection of any anticoccidial was 1.016 times more likely for every percentage increase of IFS acidic soil within the MP ZOC ( $p = 0.0212$ , 95% confidence interval is 1.002–1.030). A similar relationship with the percentage of acidic soils was observed for ionophores only ( $p = 0.0566$ ) and synthetic anticoccidials only ( $p = 0.0559$ ).

### 3.2.2 Occurrence and water quality parameters

A number of water quality parameters, as discussed below, were shown to be associated with the detection of (a) all anticoccidials, (b) ionophore anticoccidials and (c) monensin, with all other quality parameters showing no association (Supplementary File 1, Table S7). There were no relationships identified between any water quality parameters and synthetic anticoccidials. Groundwater pH and ammonium were both significantly related to the detection of ionophore compounds, while groundwater pH was also shown to be significant for detections MON alone. Results showed that for every unit increase in field pH, detection of an ionophore was 3.03 times less likely compared to a non-detection ( $p = 0.027$ , 95% confidence intervals: 1.14–8.13), while a detection of MON was 4.33 times less likely with every unit increase in pH ( $p = 0.0066$ ). Detection of an ionophore compound was 3.9 times more likely at a site that had ammonium detected compared to a site that did not have ammonium detected ( $p = 0.0266$ , 95% confidence intervals: 1.17–12.92). MON occurrence was shown to be less likely when zinc is present in the groundwater ( $p = 0.0514$ ). When accounting for confounding effects of MP type, faecal coliforms showed evidence of an effect on the detection of anticoccidial compounds ( $p = 0.066$ ). The median faecal coliform

count at sites where any anticoccidial compounds were detected was 4.7 times higher than the median faecal coliform count at the sites where no anticoccidial compounds were detected.

Again, focusing on MPs classified as “poultry present”, there was a significant association with anticoccidial detections and groundwater pH, field conductivity and calcium concentration. For every unit increase in field pH, detection of any anticoccidial was 0.15 times more likely (thus 6.7 times less likely), compared to a non-detection ( $p = 0.0183$ , 95% intervals of 0.031–0.725). For every unit increase in conductivity, detection of any anticoccidial was 1.003 times less likely, compared to a non-detection ( $p = 0.0454$ , intervals 1.0001–1.006). For every unit increase in calcium concentration, a detection of an anticoccidial was 1.016 times less likely compared to a non-detection ( $p = 0.0196$ , 95% confidence intervals: 1.003–1.030).

Ionophore compounds were related to groundwater pH ( $p = 0.0059$ ), ammonium ( $p = 0.0258$ ), calcium ( $p = 0.0334$ ) and uranium ( $p = 0.0517$ ). For every unit increase in field pH, detection of an ionophore was 12.8 times less likely, compared to a non-detection (95% intervals of 2.09–76.92), while an ionophore detection was 4.7 times more likely at MPs that had ammonium detected vs. MPs that had no ammonium. For every unit increase in calcium concentration, a detection of an ionophore was 1.014 times less likely compared to a non-detection (95% confidence intervals: 1.001–1.030). The effect observed for uranium suggested that an ionophore detection was more likely at MPs with no uranium, compared to MPs with uranium. MON was statistically related to groundwater pH ( $p = 0.0022$ ), calcium ( $p = 0.0228$ ), uranium ( $p = 0.0129$ ) and zinc ( $p = 0.0456$ ). As with the ionophores collectively, a detection of MON was shown to be less likely with every unit increase in field pH (23.6 times less likely for every unit increase in pH). Detection of MON was 6.67 times less likely (95% intervals: 1.49–29.41) at MPs where uranium was detected, compared to MPs where uranium was not detected, and 4.81 times less likely at sites that had zinc

recorded at the MPs, compared to sites that had no zinc.

## 4. Discussion

### 4.1 *Anticoccidial compounds detected*

#### 4.1.1 Ionophores

The top four most frequently detected ionophores in groundwater were MON, LAS, NAR and SAL. The trend in the compounds detected, and their frequency of detection, may be attributed to the overall usage of the compounds in Ireland, on the assumption that the primary source of these drugs in the Irish environment is as a result of the use in poultry production. These compounds make up four of the eleven anticoccidial compounds licensed in the EU for use as zootechnical feed additives in intensively reared species, under Regulations 1831/2003/EC (European Parliament, 2003). All four are licensed as feed additives intended for the control of systematic coccidiosis in different types of poultry, namely chickens reared for laying and/or chickens for fattening and/or turkeys, depending on the compound. LAS and MON are also licensed as a veterinary medicine according to Directive 2019/6/EC (European Parliament, 2019), and as listed under Commission Regulation No. 37/2010 (European Commission, 2010). However, such use of ionophores as veterinary medicines is not common in Ireland. A review of anticoccidial residues in poultry in Ireland reported that 100% of the producers used both MON and NAR, while 66% and 25% of producers reported the use of SAL and LAS respectively, for treatment of coccidiosis at their facilities (O'Keefe, 2003). The compounds detected are in relative agreement with such usage patterns, but there are other factors, as summarised below, that may influence this trend in the occurrence of ionophores.

Based on their physicochemical properties (Table 1), it is expected that ionophore compounds will be more associated with soil and sediment once in the environment, however

the extent of association of soil/sediment versus water is both pH and compound dependent (discussed in Section 4.2). Hansen et al. (2009a) give a comprehensive overview of the occurrence of ionophores in the environment, with various studies reporting the detection of ionophores in manure and manure amended soil e.g. Furtula et al. (2009) reported the detection of three ionophores (MON, NAR and SAL) and one synthetic anticoccidial (NICARB) in poultry litter, at concentrations of the order of  $\text{mg kg}^{-1}$ . While the degradation half-lives of ionophores in manure have been shown to be of the order of 4–17 days (Dolliver and Gupta, 2008, Hansen et al., 2009a), some studies have shown longer stability in stored manure for over three years (Biswas et al., 2012, Doydora et al., 2015). On application to the environment, these ionophores have the potential to persist, with MON, NAR and SAL stable under photolytic conditions, while LAS was shown to be unstable from photolysis (Bohn et al., 2013). This could explain the lower frequency of detection of LAS compared to MON, due to photodegradation prior to entering groundwater. In the same study, MON, NAR and SAL were prone to hydrolysis at pH 4 (half-lives of 0.6–13.3 days), but relatively stable under more realistic neutral and alkaline conditions. The lack of photolytic or hydrolytic degradation suggests the potential for these contaminants to persist and reach aquifers.

Sassman and Lee (2007) carried out sorption and desorption studies of MON and LAS in eight different soils and found LAS to be more associated with soil matrix, with MON shown to be more hydrophilic and associated with both aqueous and suspended solid phases. MON was also found at higher concentrations in agricultural run-off compared to sediment, a further indication of the potential mobility of MON (Davis et al., 2006). These findings are consistent with that reported by Furtula et al. (2009), with lower levels of MON associated with poultry litter, suggesting loss of MON to the solution phase during storage. Hussain and Prasher (2011) studied the sorption of MON, NAR and SAL in two wetlands and found that NAR exhibited the highest hydrophobicity, with MON being the least hydrophobic in both

soils. The higher hydrophobicity of NAR and SAL could account for the lower frequency of detection of these compounds in this current study, compared to MON.

Overall, the reported detections of ionophores, particularly MON, in run off from agricultural land (Sun et al., 2013), in surface waters (Cha et al., 2005, Kim and Carlson, 2006), and groundwaters (Watanabe et al., 2008, Bartelt-Hunt et al., 2011), indicates the relative mobility of these ionophores in the environment. All the above factors, combined with the usage discussed, and a sampling period of active groundwater recharge, provides reasonable explanation for the occurrence of these compounds.

#### 4.1.2 Synthetic anticoccidials

The most frequently detected synthetic anticoccidial in groundwater was AMP, followed by DICLAZ and NICARB. The detection and frequency of AMP in groundwater is surprising, given it is no longer authorised for use as a feed additive since its withdrawal in 2001 by Commission Regulation No. 2205/2001 (European Commission, 2001). However, it is licensed as a veterinary medicine and listed in Regulation 37/2010, for poultry species, but is therefore assumed to be present in lower amounts than the compounds used as feed additives. All other detected synthetic anticoccidials are licensed as feed additives, for treatment of coccidiosis in poultry. According to the previously mentioned O'Keefe (2003) review, 100% of producers reported the use of NICARB, as part of a combination product also containing NAR, while 51% were reported to have used DICLAZ. The review also reported that 70% of the producers used robenidine (ROB) as part of treatment, however it was not detected in groundwater as part of this study. This may be attributed to the relative instability of ROB, which has been shown to be prone to photolysis and hydrolysis at low pH, both with degradation half-lives of approx. 4 days (Hansen et al., 2009b).

AMP is relatively mobile in the environment due to its hydrophilicity, as indicated by its

water solubility ( $>500,000 \text{ mg L}^{-1}$ ) and a  $\log K_{ow}$  of -2.5 (Table 1). On assessment by EFSA, biodegradation studies carried out in five different soils indicated that AMP can persist in the environment, with degradation half-lives ranging from 60 to 417 days in the different soils (EFSA, 2018a). Further data on other degradation pathways in the environment, such as photolysis and hydrolysis, are scant for AMP. The apparent mobility and persistence of AMP, combined with its hydrophilicity, suggests the capability for AMP to be transported to groundwater, albeit at relatively low concentrations, as shown by leaching studies summarised in the EFSA assessment. Song et al. (2007; 2010) report AMP to be the most frequently detected of four pharmaceuticals, including MON, in surface water runoff from agricultural land. This study also detected AMP in different soil samples. Song et al. (2010) highlight the potential for association of the strong cationic AMP with dissolved organic matter, such as humic acids in solution, under realistic field conditions. This further suggests enhanced mobility of amprolium to groundwater, particularly in high and extreme vulnerability areas, with little soil protection, or the potential for unattenuated bypass flow. The detection of AMP in a range of hydrogeological settings (including karstic aquifers) (Supplementary File 2), combined with its high mobility, refutes the potential for the occurrences of AMP to be as a result of persistence and longer lag times. This suggests that its occurrence is potentially as a result of more recent use as a veterinary medicine, as opposed to its historical use (prior to its withdrawal in 2001) as a feed additive.

DICLAZ is licensed both as a feed additive for treating coccidiosis in poultry (primarily) and as a veterinary medicine for therapeutic use in ruminants. It exists in two forms in the environment; at low pH it remains in a neutral form and exhibits very low water solubility, high sorption and high persistence in soil, while at high pH ( $>7$ ) the anionic form has much higher solubility and much lower sorption and persistence (EFSA, 2018b). Degradation studies in soil have indicated that DICLAZ is stable at low pH with a degradation half-life of

>2000 days and a degradation half-life of 70–97 days in higher pH soils. A photolytic degradation half-life for DICLAZ was reported to be between 10 and 308 days (Hansen et al., 2009a). Given a  $pK_a$  of 5.9 for DICLAZ (Table 1) and considering that the typical pH of soils in Ireland is generally > 5.5 (Teagasc, 2018), it is unlikely for DICLAZ to be present in its fully neutral form, and therefore as the soil pH approaches and goes above 5.9, it will convert to its more mobile anionic form, allowing potential transport to groundwater. This is reflected in the common occurrence of this synthetic anticoccidial in the groundwater samples.

NICARB is an equimolar complex of 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP), which on administration, splits into the two components which are excreted separately as DNC and HDP (EFSA, 2010, EFSA, 2017). While HDP is considered to have moderate environmental mobility, due to its water solubility and low soil sorption (Table 1), aerobic stability studies in different soils have shown degradation half-lives between 3-7 days. In contrast, DNC has very poor water solubility, binds strongly to soil and has been shown to persist in different soils with half-lives ranging from 193 to 257 days under aerobic conditions. NICARB was detected in the form of DNC in the analytical method used for the groundwater analysis (Mooney et al., 2020), because of the relative instability of HDP. Potential transport of DNC to groundwater may be facilitated by colloidal transport, or sorption of the contaminants onto suspended material, which may reach the aquifer via preferential pathways, bypassing the soil matrix. Both MPs which had detections of NICARB had hydrogeological properties which might facilitate such transport i.e. one site was underlain by a regionally important karst aquifer dominated by conduit flow (solutionally widened openings) with evidence of surface-groundwater interactions, while the second MP was underlain predominantly by a diffuse flow dominated karst aquifer (Rkd) and a regionally important fractured aquifer (Rf), with groundwater within the ZOC predominantly classified as having Extreme X and E vulnerability. It must be noted however that these



observations are based on a very limited number of detections, and while the results suggest the possibility of these factors being important for the occurrence of NICARB, it is difficult to make any strong conclusion.

#### 4.1.3 Comparison to previous studies

Various ionophores and synthetic anticoccidials have been detected in surface waters and agricultural run-off (Song et al., 2010, Sun et al., 2013, Bak and Björklund, 2014), but the ionophore MON has been the only anticoccidial detected in groundwater based on literature review at the time of this study. Bartelt-Hunt et al. (2011) reported the occurrence of MON in groundwater underlying two different concentrated animal feeding operations housing cattle in the United States of America (USA). Although MON is not used in cattle in Ireland, it is authorised and used heavily as a growth promoter in cattle in the USA. Detected concentrations in groundwater at one site studied by Bartelt-Hunt et al. ranged from 180 – 2350 ng L<sup>-1</sup>, with monitoring wells downgradient of the facilities more susceptible to contamination. The second site had relatively lower MON concentrations detected (20–68 ng L<sup>-1</sup>). Watanabe et al. (2008) also reported the detection of MON in shallow groundwater underlying two different dairy facilities in the USA, with concentrations ranging from 40 to 390 ng L<sup>-1</sup>. The concentrations of MON detected in this current study (4.5–386 ng L<sup>-1</sup>) are in relative agreement with both studies, apart from the previously mentioned Bartelt-Hunt et al. study which had a higher range at one site. The ionophores LAS, NAR, and SAL, in addition to the synthetic anticoccidials AMP, DICLAZ, and NICARB were also detected in this study, the first reported detections of these anticoccidials in groundwater to the best of our knowledge in Ireland. Concentrations detected ranged from 1.9–139.9 ng L<sup>-1</sup>. There are currently no legislative limits applicable to anticoccidial compounds in groundwater or drinking water, however on application of the pesticides parametric value specified under the EU Drinking on the quality of water intended for human consumption (European

Commission, 1998), there were three sites with levels in exceedance of the 100 ng L<sup>-1</sup> individual pesticide limit. Further research is therefore needed to establish acceptable concentration ranges for these products to protect human and aquatic health.

#### *4.2 Relationship of anticoccidial occurrence with site characteristics*

##### *4.2.1 Source factors*

The occurrence of ionophore anticoccidials was significantly related to both the presence of poultry farms and poultry manure spreading within the ZOC, while the occurrence of synthetic anticoccidials was only significantly related to poultry manure spreading. This trend may be as a result of the different application and amounts used of the two groups of anticoccidials. Synthetic anticoccidials are generally used at much lower concentrations, given their better efficacy towards the parasites (Hansen et al., 2009a). Ionophores, however, are more widely used in larger amounts, because of their broad spectrum activity and slow development of resistance (Chapman et al., 2010, Chapman, 2014). There is evidence that several of the ionophores are lost from manure in solution (Section 4.1) therefore there is potential for leaching losses to groundwater, while most of the detected synthetic anticoccidials are strongly sorbed to manure, with the proposed pathways to groundwater likely via preferential flow pathways.

All but two MPs (MP008 and MP103) (Table 5) that had detections of anticoccidial compounds, were shown to have a source of poultry activity present within their ZOC. The absence of poultry at these sites is not definitive and may be due to the limitations of the data used for determining poultry activity and poultry manure spreading data. The poultry manure spreading data were based on self-declaration by the individual farmers, with access to more detailed data on poultry manure transport and usage through EPA being restricted due to the General Data Protection Regulation (European Parliament, 2016). The detections may

therefore be due to undisclosed land-spreading of poultry manure on vulnerable soils within the ZOCs of the supplies.

#### 4.2.2 Pathway factors

Pathway factors, with the exception of IFS Type I soils discussed below, were not significantly related to the occurrence of anticoccidials, however the authors believe this may be due to the scale of the investigation, which employed the predominant pathway classes within the ZOC, determined from national datasets. There is some evidence of the influence of more localised pathway factors, examples of which are discussed later in this section. There was an association ( $P < 0.06$ ) of detections with IFS Type I classification (acidic/basic soils), showing a higher likelihood of detections in groundwaters overlain by acidic soils, particularly when analysing just the MPs classified as having poultry activity present. Sorption/desorption studies for the ionophore compounds have reported  $\log K_{oc}$  to be inversely proportional to soil pH, which indicates less adsorption ( $\log K_{oc}$  decreases) to soil as pH increases (Davis et al., 2006, Sassman and Lee, 2007, Hussain and Prasher, 2011). This is likely due to the formation of anionic molecules as the soil pH approaches and increases above the  $pK_a$  of the compound, resulting in repulsion from soil surfaces. Ionophores will primarily be anionic in neutral and alkaline environments and therefore have the potential to migrate to groundwater. An interesting trend has been observed for MON whereby the  $\log K_{oc}$  increased (instead of further decrease) at higher pH ( $>7$  as reported by Sassman and Lee (2007) and  $>8.5$  by Hussain and Prasher (2011)). This effect was attributed to the complexation of MON with cationic metals and this could account for the lack of association expected in this study, between anticoccidial detections and basic soils. In higher pH soils, ionophores such as MON form lipophilic neutral complexes with metal cations such as calcium and sodium, which can consequently bind to soil, thus increasing the sorption behaviour, and reducing the mobility to groundwater.

Given the potential primary source of anticoccidials in poultry manure or litter, it is important to consider how the application can alter the behaviour of the contaminants in the receiving soil. Poultry/broiler litter is typically alkaline, with a reported pH of 8 - 9 (Nicholson et al., 1996, Doydora et al., 2015). Given the alkaline nature, there is the potential for a localised pH increase in soils receiving such manure/litter, e.g. Doydora et al. (2017) reported an overall increase of one pH unit in soil with long term broiler litter amendment, compared to unamended soils. There is also the potential for an accumulative effect if more than one type of manure is spread on the land. Whalen et al. (2000) demonstrated the effect of cattle manure application, with amended soils shown to have an increased pH, with the increase lasting for at least 8 weeks. The increase in soil pH from manure application has been attributed mainly to the presence of  $\text{CaCO}_3$  (calcium carbonate) in the manure, particularly poultry litter, however, there is also the potential of an increased pH because of the release of hydroxide ( $\text{OH}^-$ ) as a by-product of the ammonification of urea/uric acid in the manure.

A localised increase in the pH of an acidic soil of one unit would likely be enough to alter the sorption behaviour of compounds such as the ionophores, given their physicochemical properties (Table 1). In the Doydora et al. (2017) study, 46% less MON was sorbed in poultry litter amended fields, compared to unamended fields. As part of the national soil sampling carried out by Teagasc, (the Agricultural and Food Development Authority of Ireland), 55% of soils sampled (based on 45,157 samples) had a pH between 5.5 and 6.5, with just 9% having a pH of  $<5.5$  and 36% having a pH  $>6.5$  (Teagasc, 2018). At this typical pH range, several anticoccidials will be sufficiently ionised and less sorbed to the soil, therefore they have the potential to be transported to groundwater, thus reasonably explaining their occurrence in this study. Any potential localised increase in pH on the application of manure is likely to amplify such effect, with increased mobility of compounds such as DICLAZ and the ionophores, because of less sorption to soil at the higher pH. Application of manure onto

soils that are already alkaline could consequently increase the pH even further and exacerbate the complexation effects of the ionophore mentioned previously.

There was no significant relationship between anticoccidial detections and the predominant groundwater vulnerability, which is somewhat surprising given it was hypothesized that the occurrence of such contaminants would be associated with areas of higher groundwater vulnerability, because of the inherently higher susceptibility of higher vulnerability groundwaters to contamination (Daly, 2004). MPs with anticoccidial detections were relatively evenly spread across the different groundwater vulnerability classes (Figure 3 and Table 5). Notably, the percentage of detections at MPs classified as low (L) (25%) and moderate (M) (33%) was a lot higher in comparison to extreme (X) (6%) vulnerability MPs (Figure 2). Further analysis carried out on the actual percentage of each vulnerability within the ZOC (as opposed to the predominant vulnerability class) still showed no significant relationship. This suggests that the overall predominant groundwater vulnerability within the MP ZOC does not adequately reflect the potential for anticoccidial contamination at an MP, with the occurrence of these contaminants more likely accounted for on a site-specific basis, due to more localised factors.

The effect of localised factors can be illustrated with examples of two of the four MPs with anticoccidial detections that were classified as having predominantly low groundwater vulnerability. MP023 (with 67% M and L vulnerability) nevertheless has a sizeable proportion of the ZOC classified as X, E or H vulnerability (33%), and is a karst spring fed by a regionally important karst aquifer dominated by conduit flow (Rkc). This MP has several karstic solution features within its ZOC, including several sinking streams, which allow for rapid and unattenuated direct entry of contaminants to groundwater (Daly, 2000, Coxon, 2014). MP019 (BH with 99% of ZOC being L vulnerability) has a history of elevated phosphorus (P) levels in the water supply and a site report for this BH indicated the potential

for in-flow of surface runoff due to the wellhead construction (specifically a lack of protective caps), with evidence of surface ingress directly into the well. The detections of anticoccidials at the MP is therefore likely to be via the same localised pathway contributing to the high P in the BH and could potentially result from inadequate wellhead protection.

#### *4.3 Anticoccidial occurrence and water quality parameters.*

The relationship shown between the presence of ammonium in groundwater and detections of the ionophore compounds, is interesting for several reasons. Ammonium can be formed as a result of the mineralisation of organic nitrogen in poultry manure, for example, uric acid/urea can undergo ammonification to ammonium. Poultry litter also generally contains relatively high amounts of ammonia ( $\text{NH}_3$ ) and on application to acidic soils, ammonia is converted to ammonium ( $\text{NH}_4^+$ ) which will bind to negatively charged sites such as clay, in the soil (Wlazlo et al., 2016). In this regard, any ammonium that is found in groundwater is likely to have reached there by preferential flow pathways to groundwater, similar to phosphorus, which is not leached easily. Leaching studies have indicated the potential for ammonium to be leached via macropores (Silva et al., 2000). The overlying soils and subsoils also act as a natural protective layer for attenuating microbial contaminants such as faecal coliforms, with agriculture (farmyard run-off, grazing animals and land-spreading of manure and slurry) being one of the main sources of such contaminants in water (EPA, 2015). The relationship of anticoccidial detections with faecal coliforms in this study is a further indication of the importance of localised groundwater vulnerability and the potential influence of preferential secondary flow pathways on the transport of anticoccidials to groundwater. Further work would need to be carried out, possibly at the field scale, using lysimeter and leaching studies to confirm these relationships.

A significant relationship was shown between anticoccidial occurrence (particularly the ionophores) and lower groundwater pH, however, it is difficult to draw conclusion given that

the overall mean groundwater pH at sites with detections was  $6.8 \pm 0.6$  (range from 5.5–7.4), with 90% of these sites having a pH >6. Any inferences made are therefore limited to this relatively narrow pH range. At a pH >6, an appreciable fraction of most of the ionophores will be ionised and in solution phase given their  $pK_a$ 's of 4.5–6.6. This groundwater pH relationship does however agree with the relationship found between detections and IFS acidic soils. The statistical analysis also showed that detections of any anticoccidial, and the ionophores as a subgroup, were more likely in groundwaters with lower calcium and lower conductivity, with both of these trends also linked to lower pH conditions. The relationship between anticoccidial occurrence and the absence of zinc and uranium, we believe, are not causal relationships, rather are a by-product of their relationship with pH, and are more likely to be linked to the geology. However, the lack of relationship with geology, namely the bedrock units, does not strengthen this, and further investigations would need to be carried out to further explore these potential relationships.

#### *4.4 Future considerations and applicability*

Summarising the outputs of this work, the results suggest that anticoccidial drug occurrence may be more associated with MPs that have a known source of poultry and where there is evidence of contamination from poultry manure in the form of ammonium and/or faecal coliform detections, with MPs with lower groundwater pH of more interest. While these observations were shown to be statistically significant, there are a number of limitations that should be considered as part of any future application. Due to the relatively low number of detections, some of the inferences made cannot be considered as conclusive, rather should only be considered as evidence of an effect that merits further investigation. In this regard, the outputs of this work are suitable for application as a broad indicator tool only (as opposed to a predictor), for selection of potential sites for future monitoring of anticoccidial occurrence. Future work, as highlighted previously in the discussion, is required to further

investigate the relationship with groundwater ammonium.

Regarding the association of anticoccidial detections with poultry activity, it should be noted that this effect is influenced by the fact that poultry production is the primary source of anticoccidials in Ireland. This observation therefore does not imply that poultry is the only driver of anticoccidial occurrence in the environment, and on future application of the approach adopted in this work, consideration should be given to other sources of anticoccidials, which can vary from one country to another (e.g. the USA where anticoccidials are more heavily used in cattle). Finally, the authors also note that this study was carried out as part of one sampling season only during November and December 2018, which coincided with a period of active groundwater recharge. As a result, the study does not assess the potential influence of climatic effects, such as the timing of effective rainfall, on the overall occurrence of anticoccidials in groundwater. A comprehensive temporal occurrence study was beyond the scope of this work, however this is something that needs to be considered in future work, not only to assess the potential temporal variation of occurrence as a result of climatic conditions, but also to assess variations due to timing of manure application and lag times through the unsaturated zone.

## 5. Conclusions

This study, we believe, is the first of its kind to assess the occurrence of anticoccidials in groundwater, given the comprehensive suite of anticoccidials investigated, which included six ionophore and twenty synthetic anticoccidials. Up to seven different anticoccidial compounds were detected across 24% of groundwater monitoring points, at concentrations ranging from 1.9–286 ng L<sup>-1</sup>. On average, 1.7 compounds were detected at each of the 26 monitoring points with anticoccidial detections, with five monitoring points having up to three compounds detected together. Monensin, a commonly used ionophore feed additive in



poultry production, was the most frequently occurring compound, detected in 15% of all samples, while amprolium, a veterinary medicine exclusively used in poultry, was the second most frequently detected, detected in 7% of all samples.

Poultry activity was statistically shown to be a significant driver of the occurrence of anticoccidial compounds in Irish groundwater, with the occurrence of an anticoccidial 6.5 times more likely at supplies which had a source of poultry (poultry farm and/or poultry manure spreading), compared to an absence of poultry activity. Statistical analysis did not identify any clear relationships with physical site properties, however, the occurrence of any anticoccidial compounds was found to be more associated with monitoring points which contained a higher proportion of acidic soils within their zone of contribution (ZOC). Assessment of the MPs with detections and their hydrogeological properties, on a site by site basis, indicated that occurrence was likely explained by more localised factors. Several water quality parameters (groundwater pH, calcium, conductivity, ammonium, and faecal coliforms) were shown to be significantly related to the occurrence of anticoccidial compounds, or one of their subgroups (ionophores or synthetic anticoccidials). Due to the limitations of the data involved, these inferences are not definitive predictors, with further work required to confirm the relationships.

This work not only reports on the first detections of anticoccidials in an Irish groundwater context, it also reports, to the best of our knowledge, some of the first reported occurrences of lasalocid, narasin, salinomycin, amprolium, diclazuril and nicarbazin, in groundwater in Europe. Such detections indicate that these contaminants may require greater consideration in groundwater quality monitoring programmes, given that their use is anticipated to continue, if not increase, as a result of agricultural intensification. This work contributes additional information on the overall environmental groundwater occurrence of anticoccidials, thus helping to advance our understanding of their fate. The results and outputs of this work may

also provide a broad preliminary tool for the identification of potential sites for regulatory monitoring of anticoccidials, which is necessary to identify areas vulnerable to anticoccidial contamination.

## Acknowledgements

This work was carried out as part of a joint project between Trinity College Dublin and Teagasc through the Walsh Scholarship and it forms part of the Groundwater Challenge of the Irish Centre for Research in Applied Geosciences (iCRAG). This work has emanated from research supported in part by a research grant from Science Foundation Ireland (SFI) under Grant Number 13/RC/2092, co-funded under the European Regional Development Fund and by iCRAG industry partners. The LC-MS<sup>3</sup> instrumentation used for the determination of the anticoccidial drugs was funded by an SFI Research Infrastructure Grant (No. 16/RI/3710). The authors would like to express their gratitude to the following individuals and organisations who advised and/or assisted with sampling site selection and/or sampling; the Geological Survey of Ireland, the EPA Ireland, Laura Foley, and CDM Smith, Tobin, the caretakers and regional representatives of the NFGWS and staff of the Teagasc Agricultural Catchments Programme.

## References

- Alonso, L. L., Demetrio, P. M., Capparelli, A. L. & Marino, D. J. G. 2019. Behavior of ionophore antibiotics in aquatic environments in Argentina: The distribution on different scales in water courses and the role of wetlands in depuration. *Environ Int*, 133, 105144.
- Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J.-C. & García-Río, L. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agriculture, Ecosystems & Environment*, 123, 247-260.
- Bak, S. & Björklund, E. 2014. Occurrence of Ionophores in the Danish Environment. *Antibiotics*, 3, 564-571.
- Bartelt-Hunt, S., Snow, D. D., Damon-Powell, T. & Miesbach, D. 2011. Occurrence of steroid hormones and antibiotics in shallow groundwater impacted by livestock waste control facilities. *J Contam Hydrol*, 123, 94-103.
- Biswas, S., McGrath, J. M. & Sapkota, A. 2012. Quantification of ionophores in aged poultry

- litter using liquid chromatography tandem mass spectrometry. *J Environ Sci Health B*, 47, 959-66.
- Bohn, P., Bak, S. A., Bjorklund, E., Krogh, K. A. & Hansen, M. 2013. Abiotic degradation of antibiotic ionophores. *Environ Pollut*, 182, 177-83.
- Boxall, A. B. A. 2010. Veterinary Medicines and the Environment. In: Cunningham F., Elliott J. & P., L. (eds.) *Comparative and Veterinary Pharmacology. Handbook of Experimental Pharmacology*. Berlin: Springer.
- Caldeira, C., Neves, W. S., Cury, P. M., Serrano, P., Baptista, M. A. S. F. & Burdmann, E. A. 2001. Rhabdomyolysis, acute renal failure, and death after monensin ingestion. *American Journal of Kidney Diseases*, 38, 1108-1112.
- Capleton, A. C., Courage, C., Rumsby, P., Holmes, P., Stutt, E., Boxall, A. B. & Levy, L. S. 2006. Prioritising veterinary medicines according to their potential indirect human exposure and toxicity profile. *Toxicol Lett*, 163, 213-23.
- Cha, J. M., Yang, S. & Carlson, K. H. 2005. Rapid analysis of trace levels of antibiotic polyether ionophores in surface water by solid-phase extraction and liquid chromatography with ion trap tandem mass spectrometric detection. *Journal of Chromatography A*, 1065, 187-198.
- Chapman, H. D. 2014. Milestones in avian coccidiosis research: a review. *Poult Sci*, 93, 501-11.
- Chapman, H. D., Jeffers, T. K. & Williams, R. B. 2010. Forty years of monensin for the control of coccidiosis in poultry. *Poult Sci*, 89, 1788-801.
- Clabby, K. J., Bradley, C., Craig, M., Daly, D., Lucey, J., McGarrigle, M., O'Boyle, S., Tierney, D. & Bowman, J. 2008. Water Quality in Ireland (2004 - 2006): Chapter 5 Groundwater Quality in Ireland. Wexford, Ireland: Environmental Protection Agency of Ireland.
- Coxon, C. 2014. Water quality in Irish karst aquifers. *Water Resource Management: the Role of Hydrogeology, Proceedings of 34th Annual Groundwater Conference, IAH Irish Group*. Tullamore, Offaly: International Association of Hydrogeologists (Irish Group).
- Craig, M., Hunter-Williams, N. & Henning, M. 2006. Screening methodology for the Water Framework Screening methodology for the Water Framework. "It doesn't just go away, you know..." *SUDS and Groundwater Monitoring, Proceedings of the IAH (Irish Group) 26th Annual Groundwater Conference*. Tullamore, Co. Offaly, Ireland: International Association of Hydrogeologists Irish Group.
- CSO. 2017. *Census 2016 Small Area Population Statistics* [Online]. Central Statistics Office (CSO). Available: <https://www.cso.ie/en/census/census2016reports/census2016smallareapopulationstatistics/> [Accessed 5th March 2020].
- Daly, D. 2004. Groundwater at risk in Ireland – Putting geoscientific information and maps at the core of land use and environmental decision making. *John Jackson Lecture, 2004: Occasional Papers in Irish Science and Technology*, 28. Royal Dublin Society, Dublin.
- Daly, D. 2009. Groundwater—the 'Hidden Resource'. *Biology & Environment: Proceedings of the Royal Irish Academy*, 109, 221-236.
- Daly, D., Drew, D., Deakin, J., Ball, D., Parkes, M. & Wright, G. 2000. *The Karst of Ireland* [Online]. Karst Working Group-Geological Survey of Ireland Available: <http://www.gsi.ie/Programmes/Groundwater/Karst+Booklet/Water+pollution.htm> [Accessed 08/03/2016].
- Davis, J. G., Truman, C. C., Kim, S. C., Ascough, J. C., 2nd & Carlson, K. 2006. Antibiotic Transport via Runoff and Soil Loss. *J Environ Qual*, 35, 2250-60.

- DELG/EPA/GSI 1999. Groundwater Protection Schemes. Department of the Environment and Local Government, Environmental Protection Agency, Geological Survey of Ireland.
- Dolliver, H. A. & Gupta, S. C. 2008. Antibiotic losses from unprotected manure stockpiles. *J Environ Qual*, 37, 1238-44.
- Dorne, J. L., Fernandez-Cruz, M. L., Bertelsen, U., Renshaw, D. W., Peltonen, K., Anadon, A., Feil, A., Sanders, P., Wester, P. & Fink-Gremmels, J. 2013. Risk assessment of coccidiostats during feed cross-contamination: animal and human health aspects. *Toxicol Appl Pharmacol*, 270, 196-208.
- Doydora, S. A., Sun, P., Cabrera, M., Mantripragada, N., Rema, J., Pavlostathis, S. G., Huang, C. H. & Thompson, A. 2017. Long-term broiler litter amendments can alter the soil's capacity to sorb monensin. *Environ Sci Pollut Res Int*, 24, 13466-13473.
- Doydora, S. A., Sun, P., Cabrera, M., Thompson, A., Love-Myers, K., Rema, J., Calvert, V., Pavlostathis, S. G. & Huang, C. H. 2015. Stacking Time and Aluminum Sulfate Effects on Polyether Ionophores in Broiler Litter. *J Environ Qual*, 44, 1923-9.
- EFSA 2004. Update of an opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the reevaluation of coccidiostat Avatec in accordance with article 9G of Council Directive 70/524/EEC. *The EFSA Journal*. European Food Safety Authority.
- EFSA 2010. Scientific Opinion on the safety and efficacy of Maxiban® G160 (narasin and nicarbazin) for chickens for fattening. *EFSA Journal*, 8.
- EFSA 2017. Safety and efficacy of Monimax® (monensin sodium and nicarbazin) for turkeys for fattening. *EFSA Journal*, 15.
- EFSA 2018a. Safety and efficacy of COXAM® (amprolium hydrochloride) for chickens for fattening and chickens reared for laying. *EFSA Journal*, 16.
- EFSA 2018b. Safety and efficacy of Coxiril® (diclazuril) for chickens reared for laying. *EFSA Journal*, 16.
- EPA 2010. Water Quality in Ireland 2007-2009. In: McGarrigle, M., Lucey, J. & Ó Cinnéide, M. (eds.). Wexford, Ireland: Environmental Protection Agency.
- EPA 2015. Water Quality Report 2010-2012. In: Byrne, C. & Fanning, A. (eds.). Johnstown Castle, Wexford Ireland: Environmental Protection Agency (EPA).
- EPA. 2018. *Environmental Protection Agency (EPA) of Ireland GeoPortal Site* [Online]. Wexford, Ireland: Environmental Protection Agency Ireland. Available: <http://gis.epa.ie/GetData/Download> [Accessed 2019].
- EPA. 2019. *Groundwater monitoring site information-Site Folders and Maps* [Online]. Environmental Protection Agency of Ireland. Available: <https://www.epa.ie/water/wm/groundwater/gwmp/> [Accessed 2019].
- ESRI. 2016. *ArcGis ArcMap, Tools, Analysis Toolbox, Tabulate Intersection* [Online]. Environmental Systems Research Institute, Inc. Available: <https://desktop.arcgis.com/en/arcmap/10.3/tools/analysis-toolbox/tabulate-intersection.htm> [Accessed 2018].
- Essaid, H. I., Bekins, B. A. & Cozzarelli, I. M. 2015. Organic contaminant transport and fate in the subsurface: Evolution of knowledge and understanding. *Water Resource Research*, 4861-4902, 4861-4902.
- European Commission 1998. COUNCIL DIRECTIVE 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Off.J. Eur. Commun. L330/32*.
- European Commission 2001. COMMISSION REGULATION (EC) No 2205/2001 of 14 November 2001 amending Council Directive 70/524/EEC concerning additives in feedingstuffs as regards withdrawal of the authorisation of certain additives. *Off. J. Eur. Commun. L297*, 3-4.

- European Commission 2010. Commission Regulation (EU) No 37/2010 of 22nd December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of the European Union*, L15, 1-72.
- European Parliament 2003. REGULATION (EC) No 1831/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2003 on additives for use in animal nutrition. *Off. J. Eur. Commun* (2008) L268/29, L269/29-43.
- European Parliament 2006. DIRECTIVE 2006/118/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 December 2006 on the protection of groundwater against pollution and deterioration. *Off. J. Eur. Commun*. L327/19.
- European Parliament 2016. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation). *In: Off. J. Eur. Union* L119 (ed.).
- European Parliament 2019. REGULATION (EU) 2019/6 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC. *Off. J. Eur. Union*, L4, 43-55.
- Foster, S. S. D. & Chilton, P. J. 1991. Mechanisms of Groundwater Pollution by Pesticides. *Water and Environment Journal* 5, 186-193.
- Furtula, V., Huang, L. & Chambers, P. A. 2009. Determination of veterinary pharmaceuticals in poultry litter and soil by methanol extraction and liquid chromatography-tandem mass spectrometry. *J Environ Sci Health B*, 44, 717-23.
- Gill, L., O'Flaherty, V., Misstear, B. D., Brophy, L., Fennell, C., Dubber, D., O'Connell, D., Kilroy, K., Barrett, M., Johnston, P., Pilla, F. & P., G. 2018. The Impact of On-site Domestic Wastewater Effluent on Rivers and Wells. Wexford, Ireland: Environmental Protection Agency of Ireland.
- Government of Ireland 2014. European Union (Drinking Water) Regulations (S.I. No. 122 of 2014). *In: Department of Environment Community and Local Government* (ed.). Dublin 2, Ireland: The Stationary Office.
- Hansen, M., Björklund, E., Krogh, K. A. & Halling-Sørensen, B. 2009a. Analytical strategies for assessing ionophores in the environment. *TrAC Trends in Analytical Chemistry*, 28, 521-533.
- Hansen, M., Krogh, K. A., Brandt, A., Christensen, J. H. & Halling-Sørensen, B. 2009b. Fate and antibacterial potency of anticoccidial drugs and their main abiotic degradation products. *Environmental Pollution*, 157, 474-480.
- Harman, C. J., Rao, P. S. C., Basu, N. B., McGrath, G. S., Kumar, P. & Sivapalan, M. 2011. Climate, soil, and vegetation controls on the temporal variability of vadose zone transport. 47.
- Helsel, D. R. 2011. *Statistics for Censored Environmental Data Using Minitab® and R*, Hoboken, NJ, John Wiley & Sons, Inc.
- Hussain, S. A. & Prasher, S. O. 2011. Understanding the Sorption of Ionophoric Pharmaceuticals in a Treatment Wetland. *Wetlands*, 31, 563-571.
- Iglesias, A., Nebot, C., Miranda, J. M., Vazquez, B. I. & Cepeda, A. 2012. Detection and quantitative analysis of 21 veterinary drugs in river water using high-pressure liquid chromatography coupled to tandem mass spectrometry. *Environ Sci Pollut Res Int*, 19, 3235-49.
- Kelly, C. 2010. Delineating source protection zones and zones of contribution for monitoring points. *30th Annual Groundwater Conference on Groundwater in the Hydrological*



- Cycle- pressures and protection*. Tullamore, Co Offaly, Ireland: International Association of Hydrogeologists (Irish Group).
- Kim, S. C. & Carlson, K. 2006. Occurrence of ionophore antibiotics in water and sediments of a mixed-landscape watershed. *Water Res*, 40, 2549-60.
- Lapworth, D. J., Baran, N., Stuart, M. E. & Ward, R. S. 2012. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environ Pollut*, 163, 287-303.
- Long, J. S. 1997. *Regression Models for Categorical and Limited Dependent Variables*, California, USA, SAGE Publications, Inc.
- Missteart, B. D. R., Banks, D. & Clark, L. 2017. *Water Wells and Boreholes*, Chichester, John Wiley & Sons.
- Mooney, D., Coxon, C., Richards, K. G., Gill, L., Mellander, P. E. & Danaher, M. 2019. Development and Optimisation of a Multiresidue Method for the Determination of 40 Anthelmintic Compounds in Environmental Water Samples by Solid Phase Extraction (SPE) with LC-MS/MS Detection. *Molecules*, 24, 1978.
- Mooney, D., Coxon, C., Richards, K. G., Gill, L. W., Mellander, P. E. & Danaher, M. 2020. A new sensitive method for the simultaneous chromatographic separation and tandem mass spectrometry detection of anticoccidials, including highly polar compounds, in environmental waters. *Journal of Chromatography A*, 1618, 460857.
- Nicholson, F. A., Chambers, B. J. & Smith, K. A. 1996. Nutrient composition of poultry manures in England and Wales. *Bioresource Technology*, 58, 279-284.
- NSAI 2009. Irish Standard EN ISO 5667-11:2009 Water Quality Sampling Part 11: Guidance on sampling of groundwaters. National Standards Authority of Ireland.
- NSAI 2018. Irish Standard EN ISO 5667-3:2018 Water quality -Sampling Part 3: Preservation and handling of water samples. National Standards Authority of Ireland.
- O'Keefe, M. 2003. A Review of Coccidiostat Residues in Poultry. Cork, Ireland: SafeFood.
- Postigo, C. & Barcelo, D. 2015. Synthetic organic compounds and their transformation products in groundwater: occurrence, fate and mitigation. *Sci Total Environ*, 503-504, 32-47.
- Roila, R., Branciarri, R., Pecorelli, I., Cristofani, E., Carloni, C., Ranucci, D. & Fioroni, L. 2019. Occurrence and Residue Concentration of Coccidiostats in Feed and Food of Animal Origin; Human Exposure Assessment. *Foods*, 8.
- SAS Institute Inc 2014. SAS/STAT® 13.2 User's Guide. Cary, NC: SAS Institute Inc. .
- Sassman, S. A. & Lee, L. S. 2007. Sorption and degradation in soils of veterinary ionophore antibiotics: monensin and lasalocid. *Environmental Toxicology and Chemistry*, 26, 1614-1621.
- Silva, R. G., Cameron, K. C., Di, H. J., Smith, N. P. & Buchan, G. D. 2000. Effect of macropore flow on the transport of surface-applied cow urine through a soil profile. *Soil Research*, 38, 13-24.
- Song, W., Ding, Y., Chiou, C. T. & Li, H. 2010. Selected veterinary pharmaceuticals in agricultural water and soil from land application of animal manure. *J Environ Qual*, 39, 1211-7.
- Song, W., Huang, M., Rumbelha, W. & Li, H. 2007. Determination of amprolium, carbadox, monensin, and tylosin in surface water by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*, 21, 1944-50.
- Sun, P., Barmaz, D., Cabrera, M. L., Pavlostathis, S. G. & Huang, C. H. 2013. Detection and quantification of ionophore antibiotics in runoff, soil and poultry litter. *J Chromatogr A*, 1312, 10-7.
- Teagasc. 2018. *Soil Analysis Status and Trends 2018* [Online]. Johnstown Castle, Wexford: Teagasc Agricultural and Food Development Authority. Available:

- <https://www.teagasc.ie/media/website/environment/soil/All-Soil-Results-and-Enterprises-2018.pdf> [Accessed 08-04-2020 2020].
- VKM 2015. The risk of development of antimicrobial resistance with the use of coccidiostats in poultry diets. *Opinion of the the Panel on Animal Feed of the Norwegian Scientific Committee for Food Safety*. Oslo, Norway: Norwegian Scientific Committee for Food Safety (VKM).
- Watanabe, N., Harter, T. H. & Bergamaschi, B. A. 2008. Environmental occurrence and shallow ground water detection of the antibiotic monensin from dairy farms. *J Environ Qual*, 37, S78-85.
- Whalen, J. K., Chang, C., Clayton, G. W. & Carefoot, J. P. 2000. Cattle manure amendments can increase the pH of acid soils. *Soil Science Society of America Journal*, 64 (3), 962-966.
- Wlazlo, L., Nowakowicz-Debek, B., Kapica, J., Kwiecien, M. & Pawlak, H. 2016. Removal of ammonia from poultry manure by aluminosilicates. *J Environ Manage*, 183, 722-725.
- Working Group on Groundwater. 2001. *Technical Requirements for Groundwater and related aspects- Guidance Document GW1* [Online]. Available: <http://www.wfdireland.ie/Documents/Characterisation%20Report/Background%20Information/Analysis%20of%20Characters/Groundwater/GW1%20Technical%20Requirements.pdf> [Accessed].
- Yopasá-Arenas, A. & Fostier, A. H. 2018. Exposure of Brazilian soil and groundwater to pollution by coccidiostats and antimicrobial agents used as growth promoters. *Sci Total Environ*, 644, 112-121.

## Figure Captions

**Figure 1.** The spatial distribution of the 109 sampling monitoring points, classified as boreholes (BH) vs. springs (SP) and detection (red) vs. non-detection (green), overlaid onto the GSI national bedrock aquifer Map (GSI, 2015b & 2015c)

**Figure 2 (a):** summary of the number of sites within each of the GSI aquifer category, WFD flow regime and groundwater vulnerability classes, that had detections and **(b)** the percentage (%) of sites within each category that had detections, broken down into sites with poultry activity present vs. absent.

**Table 1.** Chemical and physicochemical data of the 26 anticoccidial compounds (grouped by class), investigated in the spatial occurrence study, with corresponding analytical method performance parameter

[illegible]



Semduramic	SEMD	C <sub>4</sub> H <sub>7</sub> O <sub>16</sub>	11337	1.0 - 250	0.25	1	163 -	2.6	5.4	1.4
in*			8-31-7				1240			-
										3.3

**Synthetic****Anticoccidials**

Aklomide	AKLO	C <sub>7</sub> H <sub>5</sub> ClN <sub>2</sub> O <sub>3</sub>	3011- 89-0	20.0 - 250	5	20	-	-	-	-
Amprolium	AMO	C <sub>14</sub> H <sub>19</sub> N <sub>4</sub> Cl	125- 25-2	0.5 - 250	0.1	0.5	540,32 0	-2.5	4.65	3- 3.7
ANOT	ANOT	C <sub>8</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	3572- 44-9	10.0 - 150	2.5	10	-	-	-	-
Arprinocid	ARPRIN	C <sub>12</sub> H <sub>9</sub> ClFN <sub>5</sub>	55779- 18-5	0.5 - 150	0.1	0.5	-	-	-	-
Buquinolate	BUQUIN	C <sub>20</sub> H <sub>27</sub> NO <sub>5</sub>	5486- 03-3	0.5 - 150	0.1	0.5	-	-	-	-
Clopidol	CLOP	C <sub>7</sub> H <sub>7</sub> Cl <sub>2</sub> NO	2971- 90-6	0.5 - 150	0.1	0.5	-	-	-	-
Cyromazine	CYROM	C <sub>6</sub> H <sub>10</sub> N <sub>6</sub>	66215- 27-8	1.0 - 250	0.1	1	8000 - 13000	0.069	5.22	-
Decoquate	DECO	C <sub>24</sub> H <sub>35</sub> NO <sub>5</sub>	18507- 89-6	0.5 - 150	0.1	0.5	0.06	5.2- 5.5	-	>5. 6
Diaveridine	DIAB	C <sub>13</sub> H <sub>16</sub> N <sub>4</sub> O <sub>2</sub>	5355- 16-8	0.5 - 150	0.15	0.5				-
Diclazuril*	DICLAZ	C <sub>17</sub> H <sub>9</sub> Cl <sub>3</sub> N <sub>4</sub> O <sub>2</sub>	10183 1-37-2	0.1 - 250	0.02	0.1	1.44 - 2.6	3.6	5.89	3.7 - 4.0
Dinitolmide	DINITOL	C <sub>8</sub> H <sub>7</sub> N <sub>3</sub> O <sub>5</sub>	148- 01-6	10.0 - 150	2	10	-	-	-	-
Ethopabate	ETHO	C <sub>12</sub> H <sub>15</sub> NO <sub>4</sub>	59-06- 3	0.1 - 250	0.02	0.1	-	-	-	-
Halofuginon	HALO	C <sub>16</sub> H <sub>17</sub> BrClN <sub>3</sub>	55837- 20-2	0.1 - 250	0.05	0.1	3000	1 - 2.5	-	-
Nequate	NEQUIN	C <sub>22</sub> H <sub>23</sub> NO <sub>4</sub>	13997-	0.5 - 150	0.1	0.5	-	-	-	

19-8										
Nicarbazin* (DNC) <sup>b</sup>	NICARB	C <sub>13</sub> H <sub>10</sub> N <sub>4</sub> O <sub>5</sub>	330- 95-0	1.0 - 250	0.1	1	<0.02	>3.6	12.4	4.2 4 5.1
Nicarbazin (HDP)		C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> O					>60,00 0	-0.94	3.75	1.5 - 2.2
Nitromide	NITRO	C <sub>7</sub> H <sub>5</sub> N <sub>3</sub> O <sub>5</sub>	121- 81-3	20.0 - 250	5	20	-	-	-	-
Robenidine *	ROB	C <sub>15</sub> H <sub>13</sub> Cl <sub>2</sub> N <sub>5</sub>	25875- 51-8	0.1 - 250	0.03	0.1	118	3.3	3.4	5.6
Toltrazuril	TOL	C <sub>18</sub> H <sub>14</sub> F <sub>3</sub> N <sub>3</sub> O <sub>4</sub>	69004- 03-1	20.0 - 250	4	20	-	-	-	-
Toltrazuril sulphone	TOL-SO <sub>2</sub>	C <sub>18</sub> H <sub>14</sub> F <sub>3</sub> N <sub>3</sub> O <sub>6</sub>	69004- 04-2	20.0 - 250	10	20	1	2.49	7.15	2.8
Toltrazuril sulphoxide	TOL-SO	C <sub>18</sub> H <sub>14</sub> F <sub>3</sub> N <sub>3</sub> O <sub>5</sub>	69004- 15-5	20.0 - 250	4	20	-	-	-	-

\* Compounds licensed as feed additives in the European Union under Regulations 1831/2003/EC (European Parliament,

2003)

<sup>a</sup> Calibration

range, LOD and LOQ data taken from (Mooney et al. 2020) <sup>b</sup> Nicarbazin detected as DNC <sup>c</sup> physicochemical data extracted from the EFSA Journal (EFSA, 2020)

LOD = Limit of detection, LOQ = Limit of quantification, S<sub>w</sub> = water solubility, logK<sub>ow</sub> = logarithm of octanol-water

partition coefficient, pK<sub>a</sub> = dissociation constant and logK<sub>oc</sub> = logarithm of soil organic carbon-water partitioning coefficient

**Table 2** Summary of some of the main land use and physical hydrogeological site properties, used to characterise sampling MPs for site selection and statistical analysis, with the corresponding national dataset source

Prope rtv**	MP Type	Corine	Bedrock Geology	Aquifer	WFD	Ground water vulnera bility*	Irish		Quate rnary Sedim ents	Subsoil Permea bility
		Land Cover		Category *	Flow Regime*		Fore stry Soils (IFS)	SIS Irish Soils		

Data source	EPA	Corine	Hydrostrat	Groundwater	Amalgamated from	Groundwater	IFS	The Irish Soils Information System	Quarterly	Groundwater
Ref		Land Cover 2012	igraphic Rock Units	ater Bedrock	ated from GSI	ater Vulnerability	National Soil	Soils Informatio n System	nary Sedime nts	water Subsoil
		Digital Map	Group Map	Map 1:100,000	Categorie s	Map1:40 ,000	Map 1:50, 000	National Map	Map 1:50,00 0	Permeab ility
			1:100,000 (Digital)	& Gravel		(Digital) GSI	000 (Digi tal)	1:250,000 (Digital)	0 (Digital)	Map 1:40,00 0
			GSI	Aquifers 1:50,000( Digital)			from the EPA	EPA	) GSI	0 (Digital) GSI
	(EPA, 2011)	(EPA, 2012)	2016a) (Tedd et al. 2017)	2015b) (GSI, 2015c)	(WGGW, 2001)	(GSI, 2015d)	(Teagasc-EPA-GSI, 2006)	(Teagasc-EPA, 2014)	(GSI, 2016b)	(GSI, 2015a)
Classe s	• Bor ehole	• Corine (level 3): ◦ See Supplementary file 1 • Corine (amalgamated) ◦ Arabic ◦ non arabic (pasture) ◦ forest ◦ other	27 rock units amalgamated into six lithological groups • Sand and gravel • Impure limestone • Pure limestone • Non-	11 classes as follows: • Regionally Important ◦ Rk ◦ Rkc ◦ Rkd ◦ Rf ◦ Rg • Locally Important ◦ Lm ◦ Lk ◦ Ll ◦ Lg • Poor Aquifer ◦ Pl ◦ Pu • Intergranular ◦ (Rg	• Karstic ◦ Rk, RKc, Rkd & Lk • Productive fractured ◦ Rf & Lm • Poorly productive ◦ Ll, Pl and Pu • Intergranular ◦ (Rg	• X-Extreme (exposed)  • E – Extreme  • H-High  • M-Moderate  • L-Low	Type I: Acid vs. Base  Type II: Mine ral vs Peat  Type III: Deep	• Soil Association -61 (see supplementary)  • Drainage ◦ Excessive ◦ Well ◦ Moderate ◦ Imperfect ◦ Poor  • Texture	(Genes is)  • Alluvium  • Irish Sea Tills  • Karstified rock  • Peat  • Sand and Gravels  • Tills	• High  • Mode rate  • Low  • DTB< 3m ***

- Bedrock at surface

Type

#### IV:

Dry

Rk = Regionally Important Aquifer-Karstified, Rkc = Regionally Important Aquifer-Karstified (conduit flow), Rkd = Regionally Important Aquifer-Karstified (diffuse flow), Rf = Regionally Important Aquifer – Fissured bedrock, Lm = Locally Important Aquifer – Bedrock which is Generally Moderately Productive, Lk = Locally Important- Karstified, Ll = Locally Important Aquifer - Bedrock which is Moderately Productive only in Local Zones, Pl = Poor Aquifer - Bedrock which is Generally Unproductive except for Local Zones and Pu = Poor Aquifer - Bedrock which is Generally Unproductive Rg = Regionally Important Gravel Aquifers, Lg = Locally Important gravel aquifer

**Table 3.** The relative proportion of the 109 sampling sites, subdivided into poultry present vs. absent, spread across nine GSI bedrock aquifer classifications, three WFD bedrock aquifer flow regime categories and the groundwater vulnerability categories

Poul try Acti vity*	N o. Si te s	Aquifer Category (GSI Classification) **										WFD Flow Regime***		Groundwater Vulnerability****				
		R k c	R k d	L k f	R m	L l	P l	P u	Kar stic	Prod uctive Fract ured	Poorl y Prod uctive	Extr eme (X)*	Extr eme (E)	Hi gh (H)	Mod erate (M)	L ow (L)		
Prese nt*	74	1	2	18	1	3	6	1	4	1	41	10	23	7	13	29	13	12
Abse nt*	35	0	7	6	0	7	2	8	4	1	13	9	13	9	7	10	5	4

Total	10	1	2	24	1	1	8	2	8	2	54	19	36	16	20	39	18	16
	9		9			0		6										
% of total sites	-	1	2 7	22	1	9	7	2 4	7	2	50	17	33	15	18	36	17	15

\* Presence/Absence of poultry activity according to DAFM dataset only, with a “poultry activity” being a poultry farm and/or poultry manure spreading activity.

\*\* Rkc = Regionally Important Aquifer-Karstified (dominated by conduit flow), Rkd = Regionally Important Aquifer-Karstified (dominated by diffuse flow), LI = Locally Important Aquifer - Bedrock which is Moderately Productive only in Local Zones, PI = Poor Aquifer - Bedrock which is Generally Unproductive except for Local Zones and Pu = Poor Aquifer - Bedrock which is Generally Unproductive

\*\*\*Groundwater flow regime classified by grouping GSI aquifer categories, where karstic = Rk, Rkc, Rkd and Lk, Productive Fractured = Rf and Lm while Poorly Productive = LI, PI and Pu

\*\*\*\* Extreme-X = rock at or near surface or karst

**Table 4.** Summary statistics for the seven anticoccidial compounds detected above the limit of quantification (LOQ)

Analyte	No. samples > LOQ	% Samples > LOQ	% of Total Detections	Concentration (ng L <sup>-1</sup> )			
				Min	Max	Mean	Median
<b><u>Ionophores**</u></b>	21	19.3	81	-	-	-	-
Lasalocid (LAS)	6	5.5	23.1	1.9	55.7	19.5	14.7
Monensin (MON)	16	14.7	61.5	4.5	385.7	47.1	17.5
Narasin (NAR)	4	3.7	15.4	6.5	46.7	19.1	11.6
Salinomycin (SAL)	4	3.7	15.4	6.5	18.6	11.2	9.8
<b><u>Synthetic Anticoccidials**</u></b>	12	11	42	-	-	-	-
Amprolium (AMP)	8	7.3	30.8	2.8	49.8	14.5	11.9
Diclazuril (DICLAZ)	5	4.6	19.2	3.7	65.6	21.8	14.7
Nicarbazin (NICARB)	2	1.8	7.7	29.5	134.9	82.2	82.2

\* LOQ = Limit of Quantification, see Table 1 for LOQ values for individual analytes

\*\* Data presented for ionophores collectively and synthetic anticoccidials collectively, indicates the number of sites (and respective percentages) which had detection of one or more ionophores/ one or more synthetic anticoccidials at concentrations greater than the LOQ.

**Table 5.** Summary of the anticoccidial compounds detected, and corresponding concentrations, at each of the 26 sites that had a detection recorded

M P N o.	MP Typ e	Regi on	Aqui fer Class *	Flow Regim e*	Groundw ater Vulnerabi lity*	Poult ry Activ ity	Analyte, Mean Concentration (ng L <sup>-1</sup> ) (n = 3 replicates)						
							LA	MO	SA	NA	A	DICL	NICA
							S	N	L	R	MP	AZ	RB
16	BH	Border	Rf	Productive Fractured	H	Present	5.2	n.d.	6.5	n.d.	n.d.	3.7	n.d.
19	BH	Border	Rkd	Karstic	L	Present	n.d.	39.2	n.d.	n.d.	n.d.	n.d.	n.d.
38	BH	Border	Rk	Karstic	M	Present	24.2	n.d.	n.d.	n.d.	13.7	14.7	n.d.
90	BH	Border	Rkc	Karstic	M	Present	n.d.	n.d.	n.d.	n.d.	n.d.	16.0	134.9
108	BH	Border	Ll	Poorly Productive	L	Present	n.d.	112	n.d.	15.1	10.8	n.d.	n.d.
109	BH	Border	Ll	Poorly Productive	M	Present	n.d.	24.0	n.d.	n.d.	n.d.	n.d.	n.d.
138	BH	Mid-East	Rkd	Karstic	H	Present	n.d.	14.7	n.d.	6.5	n.d.	n.d.	n.d.
8	BH	Midland	Rf	Productive Fractured	H	Absent	n.d.	385.7	n.d.	46.7	n.d.	n.d.	n.d.
50	BH	Midland	Ll	Poorly Productive	H	Present	n.d.	11.7	n.d.	n.d.	n.d.	n.d.	n.d.
59	Spring	Mid-West	Ll	Poorly Productive	E	Present	n.d.	n.d.	n.d.	n.d.	49.8	n.d.	n.d.
14	BH	South-East	Pl	Poorly Productive	E	Present	n.d.	5.6	n.d.	n.d.	n.d.	n.d.	n.d.
72	BH	South-East	Rkd	Karstic	M	Present	n.d.	12.8	n.d.	8.1	n.d.	n.d.	n.d.
85	BH	South-East	Rkd	Karstic	E	Present	n.d.	20.3	n.d.	n.d.	n.d.	n.d.	n.d.
103	BH	South-East	Rf	Productive Fractured	X	Absent	n.d.	n.d.	n.d.	n.d.	5.1	9.3	n.d.
107	BH	South-East	Rkd	Karstic	H	Present	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29.5
79	BH	South-West	Ll	Productive Fractured	E	Present	55.7	4.5	n.d.	n.d.	n.d.	n.d.	n.d.
96	Spring	South-West	Ll	Poorly Productive	H	Present	n.d.	9.0	n.d.	n.d.	n.d.	n.d.	n.d.

97	Spring	South-West	Ll	Poorly Productive	H	Present	25.5	n.d.	n.d.	n.d.	14.3	n.d.	n.d.
9	BH	West	Pl	Poorly Productive	E	Present	n.d.	44.8	18.6	n.d.	6.2	n.d.	n.d.
23	Spring	West	Rkc	Karstic	L	Present	n.d.	22.7	7.9	n.d.	2.8	n.d.	n.d.
32	BH	West	Ll	Poorly Productive	L	Present	n.d.	11.5	n.d.	n.d.	n.d.	n.d.	n.d.
45	Spring	West	Rkc	Karstic	E	Present	n.d.	27.4	11.8	n.d.	n.d.	n.d.	n.d.
58	Spring	West	Rkc	Karstic	M	Present	1.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
66	Spring	West	Rkc	Karstic	E	Present	n.d.	n.d.	n.d.	n.d.	12.9	n.d.	n.d.
70	Spring	West	Rkc	Karstic	M	Present	4.4	n.d.	n.d.	n.d.	n.d.	65.6	n.d.
71	Spring	West	Ll	Poorly Productive	H	Present	n.d.	7.1	n.d.	n.d.	n.d.	n.d.	n.d.

\*Predominant class within the zone of contribution, MP = monitoring point, BH = borehole, SP = spring, Border region = counties Cavan, Donegal, Leitrim, Louth and Monaghan

**Table 6.** Summary of site characteristics showing a significant relationship with the occurrence of anticoccidials, defined as all anticoccidials, ionophores and synthetic anticoccidials, with corresponding p-values, confidence intervals and odds ratio likelihood interpretations

Site	Detection	Odds	95%	p-value	Odds ratio interpretation
Characteristic	defined as:	ratio	intervals		
<b>Poultry activity</b>	All anticoccidials	6.5	1.62 – 26.32	0.0083	6.5 times more likely to have “any anticoccidial” detection with poultry activity present in the ZOC, compared to when poultry activity is absent
	Ionophores	8.6	1.53 – 50.00	0.0148	8.6 times more likely to have an ionophore detection with poultry activity present in the ZOC, compared to when poultry activity is absent
	Synthetic anticoccidial	-	-	0.1150	Not significant
<b>Poultry farm density</b>	All anticoccidials	4.6	2.04 – 10.25	0.0002	4.6 times more likely to have “any anticoccidial” detection than not, for every unit increase in poultry farm density within the ZOC
	Ionophores	4.9	2.14 – 11.39	0.0002	4.9 times more likely to have an ionophore detection than not, for every unit increase in poultry farm density within the ZOC
	Synthetic anticoccidial	-	-	0.3555	Not significant
<b>Poultry manure</b>	All anticoccidials	9.3	2.62 – 32.26	0.0005	9.3 times more likely to have “any anticoccidial” detection with poultry manure spreading in the ZOC, compared to no poultry manure spreading
	Ionophores	7.3	2.01 – 26.31	0.0025	7.3 times more likely to have an ionophore detection with poultry manure spreading in the ZOC, compared to no poultry manure spreading
	Synthetic anticoccidial	6.1	1.66 – 22.73	0.0065	6.1 times more likely to have a synthetic anticoccidial detection with poultry manure spreading in the ZOC, compared to no poultry manure spreading



<b>Irish Forestry Soils (IFS) acidic/basic</b>	All anticoccidials	4.0	0.978 – 6.589	0.0555	4.0 times more likely to have a synthetic anticoccidial detection in acidic soils, compared to basic soils *marginal significance*
	Ionophores	-	-	0.1327	Not significant
	Synthetic anticoccidial	7.2	1.24 – 42.11	0.0282	7.2 times more likely to have a synthetic anticoccidial detection in acidic soils, compared to basic soils

---

### **Declaration of competing interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**CRedit author statement**

**Damien Mooney:** Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing – Original Draft, Visualization, Project Administration. **Karl Richards:** Conceptualization, Resources, Writing – Review and Editing, Supervision, Funding Acquisition. **Martin Danaher:** Conceptualization, Methodology, Resources, Writing – Review and Editing, Visualization, Supervision, Funding Acquisition. **Jim Grant:** Software, Formal Analysis, Writing – Review and Editing. **Laurence Gill:** Conceptualization, Writing – Review and Editing, Funding Acquisition. **Per-Erik Mellander:** Conceptualization, Resources, Writing – Review and Editing. **Catherine Coxon:** Conceptualization, Writing – Review and Editing, Visualization, Supervision, Project Administration, Funding Acquisition.

## Graphical Abstract

### Highlights

- Anticoccidial veterinary drugs are emerging groundwater contaminants of concern
- Sites sampled for anticoccidials accounted for land-use and hydrogeological factors
- Seven of twenty-six compounds were detected, most frequently the ionophore monensin
- Amprolium and other coccidiostats are documented in groundwater for the first time
- Poultry activity is a significant driver of anticoccidial occurrence in groundwater

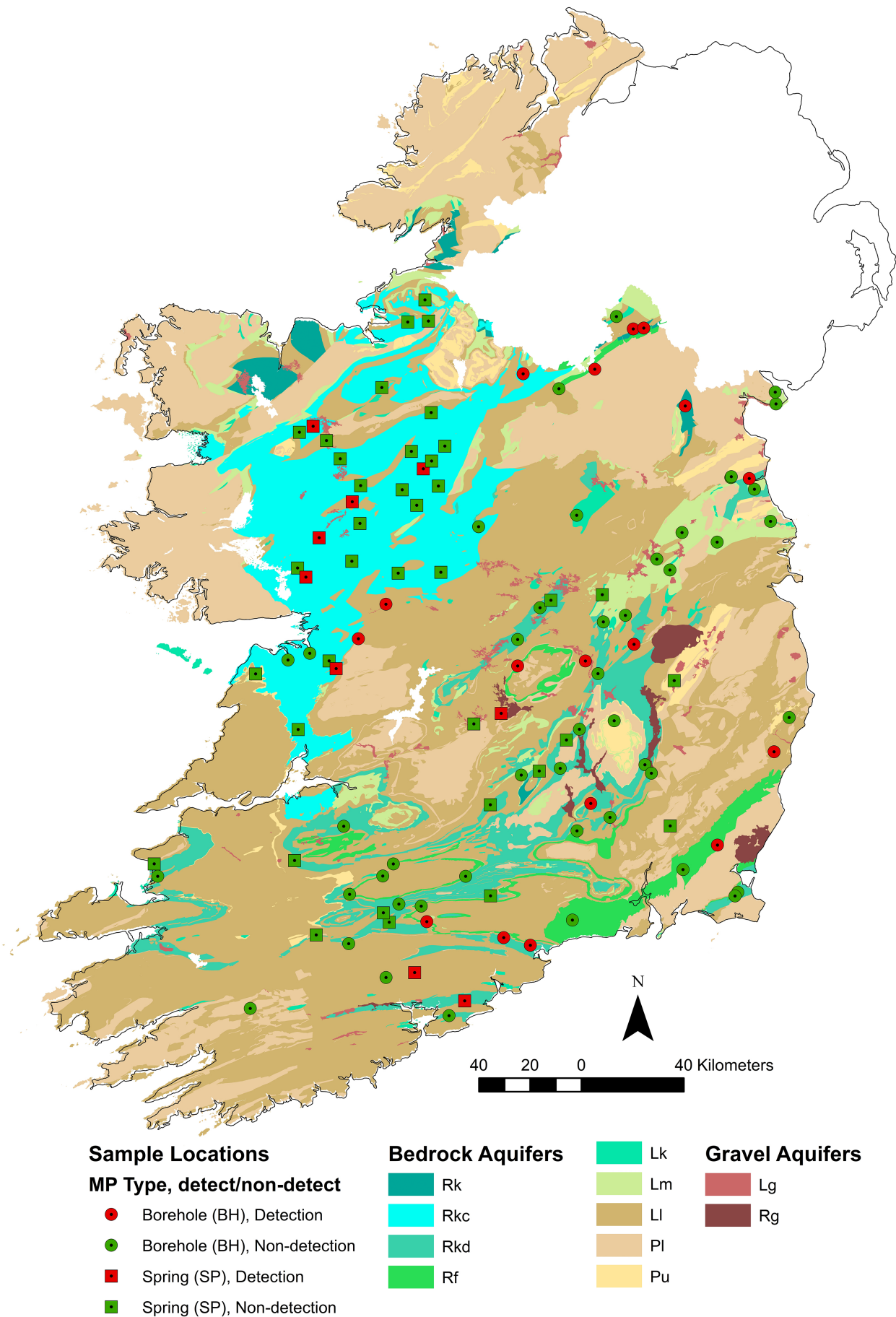


Figure 1

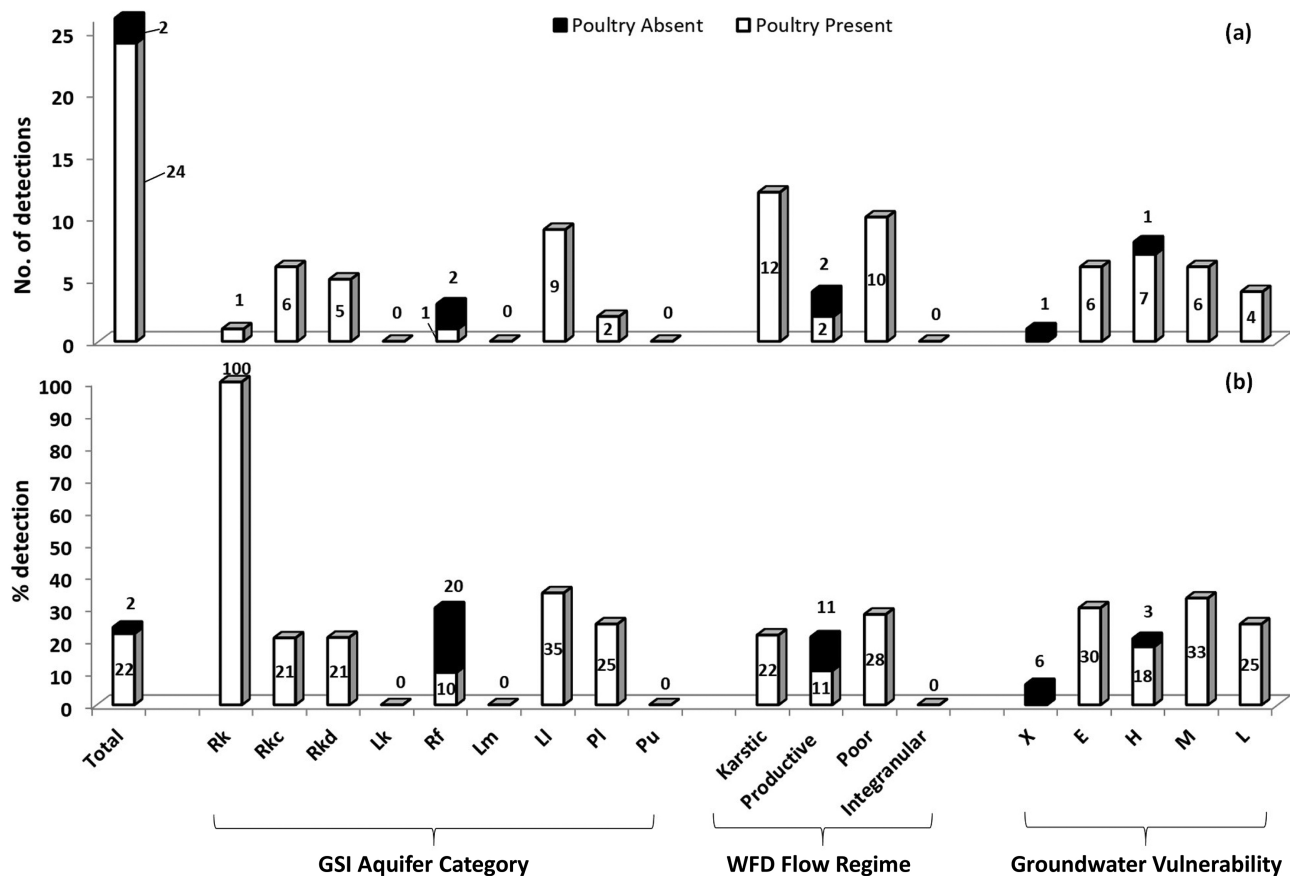


Figure 2